INVESTOR IN PEOPLE

PRIORITY

DOCUMENT

SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

REC'D 1 4 JUN 2004

WIPO   PCT

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.
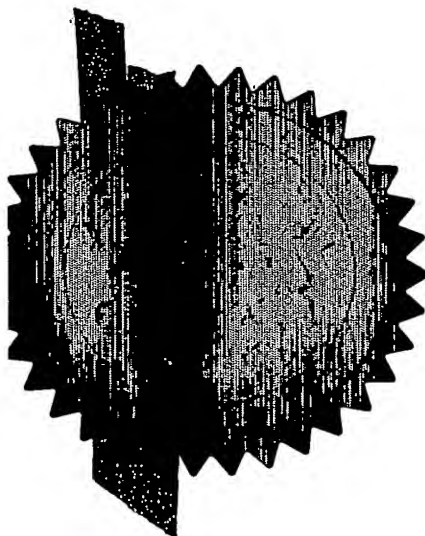
Signed   R. Mahoney

Dated   3 June 2004

Patents Form 1/77

Patents Act 1977
(Rule 16)

The Patent Office

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

[stamp: THE PATENT OFFICE H 12 MAY 2003 LONDON]

1. Your reference — GBP87955

2. Patent application number
(The Patent Office will fill in this part)
: **0310880.0**

1 2 MAY 2003

3. Full name, address and postcode of the or of each applicant (underline all surnames)

Oxitec Limited,
2nd Floor
Park Gate
25 Milton Park
Oxford OX14 4SH
United Kingdom

Patents ADP number (if you know it)

8571051001

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

4. Title of the invention — RESISTANCE DILUTION

5. Name of your agent (if you have one)
"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

Marks & Clerk
57 - 60 Lincolns Inn fields
London WC2A 3LS

Patents ADP number (if you know it)

18001 ✓

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country	Priority application No (if you know it)	Date of filing (day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing (day / month / year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

9. Enter the number of sheets for any of the
   following items you are filing with this form.
   Do not count copies of the same document

Continuation sheets of this form	0
Description	32
Claim(s)	2
Abstract	1
Drawing(s)	0

10. If you are also filing any of the following,
    state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent (Patents Form 7/77)	
Request for preliminary examination and search (Patents Form 9/77)	1
Request for substantive examination (Patents Form 10/77)	
Any other documents (please specify)	

11.   I/We request the grant of a patent on the basis of this application.

Signature B.South   Date: 12 May 2003

12. Name and daytime telephone number of
    person to contact in the United Kingdom

Patent Chemical Formalities
020 7400 3000

RESISTANCE DILUTION

The present invention relates to breeding strategies for reducing the frequency of, or eliminating, genetic traits, such as pesticide resistance, in a target population.

Ways of controlling pests, and especially those which attack important harvests or which are capable of spreading disease, are continually being sought. With each new strategy that is found for eliminating or neutralising a pest, comes the concern that the pest in question will develop resistance, or become immune, to the strategy.

For example, the Cry1Ac protein from *Bacillus thuringiensis* can successfully be expressed in many important crops, and is effective as a pesticide, both when used as a spray or when expressed by the plants themselves. However, instances of resistance to *Bt* toxin have already been noticed amongst the diamondback moth population, for example. As resistance spreads, alternative strategies for controlling pest populations will have to be found.

Resistance genes generally confer a level of selective disadvantage. If this were not the case, then the genes would normally be present at substantial levels in the population already, and would spread exceedingly rapidly, once use of the toxin became commonplace. Such genes may have their effect by blocking a particular pathway or preventing a particular substance crossing the cell membrane, for example.

Even at the simplest level, where only one locus is associated with resistance, most resistance genes show some degree of partial or co-dominance. Thus, pests which are heterozygous for the resistance gene still show some resistance to the toxin, especially at low levels of toxin. This is not always the case, and some *Bt* resistance, for example, appears to be purely recessive, *i.e.* if the insect is not homozygous for resistance, then there is no resistance.

Accordingly, the spread of resistance is often difficult to spot, as insects only heterozygous for the resistance gene will show only some selective advantage in areas

where the pesticide is used and, by the time that homozygous insects start to be commonplace, a large proportion of the population will contain at least one resistant gene, so that the effect is effectively for a resistant population to appear "out of nowhere".

Various strategies are employed for minimising pesticide resistance. Commonly used strategies include: endeavouring to minimise use, thereby reducing the selective pressure for development of resistance; the use of different toxins in combination, as well as developing and using new pesticides, so as not to encourage resistance to develop against any one particular pesticide; the use of toxins at high concentration formulation to increase the effect on heterozygotes or weakly resistant individuals; and the use of pesticides within an integrated pest management framework.

Other methods for reducing the pest population size, such as using the conventional Sterile Insect Technique (SIT), can also have the effect of increasing the level of inbreeding, which can increase the rate at which homozygous resistant genotypes appear. A particular problem associated with this technique lies in the fitness, or competitiveness, of the sterile insects, which can be as low as a tenth that of the wild type. Given that the quantity of the next generation eliminated by this technique can only be as high as 50%, for 100% release by numerical comparison with the wild population, it is necessary to release numbers in excess of several times the indigenous population in order to have a significant effect.

More recently, especially with the use of *Bt* crops, the "high dose/refuge strategy" has started to be employed. In this strategy, a "refuge" of non-transformed plants is provided, together with the *Bt* plants. The purpose of this is to encourage the proliferation of sensitive, or wild-type, insects in the refuge areas, so that the sensitive genes will continue to be encouraged and spread into the resistant insects to dilute the onset of resistance. The "high dose" relates to the plants, which provide a sufficiently high dose of the *Bt* toxin to kill off any insects which are only heterozygous for resistance, thereby forcing the resistance to become a recessive trait, *i.e.* only insects homozygous for the resistance gene will survive. Sensitive genes carried by the insects breeding in the refuges then serve to generate heterozygotes which cannot survive the

high dose plants. The use of the high dose/refuge strategy does not preclude the complementary use of pesticides, or other pest control methods.

Population models show that this strategy should greatly restrict the spread of resistance, but will generally only be effective to slow it down. Even with refuge areas as high as 50%, the complete spread of resistance may only delayed by as little as 10 years, after which substantial or complete resistance can be expected to have rendered the toxin useless.

In addition, end user compliance is likely to be exceedingly difficult to enforce, as it is not in the immediate commercial interests of a farmer to use no pest control strategy, or a sub-optimal one, on a significant percentage of his crop, hence potentially allowing 20% or more of his crop to be destroyed by pests, simply in order to continue to be able to use that same toxin in 10 years time. Although this effect can be ameliorated by the use of other pesticides on the refuges, there will remain a significant temptation not to plant refugia.

There is also the possibility of employing a "non-high dose/refuge" strategy, such as has recently been proposed in relation to an anti-corn root worm product, where some experts considered that the appropriate refuge size should be 50%, as the level of *Bt* toxin expressed only kills about half of the root worm larvae. Any resistance could, therefore, be expected to spread rapidly. However, a refuge of 50% is unlikely to be commercially acceptable in many quarters, which has lead to calls for a 20% refuge that may be less effective at halting the spread of resistance.

WO 01/39599 discloses a method for controlling the population of a pest, such as an insect, by the release of insects carrying dominant lethal genes. The dominant lethal gene is suppressible under controlled conditions but, on release, the lethal phenotype is expressed. In order for this RIDL release to continue to be effective, the dominant lethal is generally sex specific, and is generally chosen to kill all female progeny. With time, however, resistance or tolerance can arise to any specific genes used in the RIDL technique.

At present, once resistance begins to appear, the best that can be done is to slow down the spread of resistance, with the only guaranteed way to stop resistance spreading any further being the cessation of use of the toxin, or pesticide. There is a clear need for further methods for slowing the spread of resistance and, particularly, for preventing or even reducing or eliminating established resistance.

Surprisingly, it has now been found, where a pest reproduces sexually, and especially where carriers of resistance genes have lesser fitness than the wild type under conditions not selective for resistance, then simple introduction of the wild type into a population is sufficient to reduce or prevent the spread of resistance and, at high enough levels, is even capable of eliminating established resistance.

Thus, in a first aspect, the present invention provides a method for the inhibition or reversal of the spread of a non wild type genetic trait in a non human population capable of sexual reproduction, the trait conferring a reduced level of fitness of the individuals carrying that trait in either the heterozygous or homozygous form, the method comprising introducing sexually compatible individuals substantially homozygous for the wild type counterpart of the trait into the target population.

This method is distinct from the simple use of refuges, or refugia, in that the individuals released into the target population are guaranteed to be free, or substantially free, of resistance. By contrast, with the use of refugia, there is no selection against the resistance phenotype other than the relative fitness of the genotype it carries. Thus, typically, greater and greater levels of insects carrying at least one copy of the resistance gene will form the population characterising the refugia so that, eventually, resistance will become the norm.

By contrast, the method of the present invention guarantees a pure, or substantially pure, influx of individuals homozygous for the sensitive gene, thereby diluting the resistance gene pool. With continued use of the method, the simple fact that individuals carrying resistance genes have reduced levels of fitness in the absence of the selective agent, combined with simple dilution of the resistance allele, will restrict, prevent, or even reverse the spread of resistance.

Individuals heterozygous for the resistance gene(s) generally have much lower fitness on the toxin than homozygotes which, combined with the dilution effect that greatly reduces the number of resistant homozygotes, tends to reduce the overall fitness of the resistance allele. This effect results not only from reducing the allele frequency, but also by preventing the population reaching Hardy-Weinberg equilibrium. With continued use of the method, the simple fact that the released individuals do not, or predominantly do not, carry resistance alleles, and so tend to dilute the resistance alleles within the population, and particularly the relative frequency with which homozygous resistant individuals arise, will restrict, prevent, or even reverse the spread of resistance.

Suitable populations will generally be wild populations, such as mosquitoes, for example, but may be any suitable population in which it is desired to control the spread of a non wild type trait, or trait where an increased fitness alternative can be used to control the trait. Cultivated plant crops may develop abnormal growth, for example, and release of appropriate pollen may be used to eliminate or reduce further incidence.

By "sexually compatible" is meant that the individuals introduced into the population are capable of sexual reproduction with members of the target population. The individuals are preferably of the same species, but this is not essential, provided that fertile or partially fertile offspring are possible. Where the introduced individuals are essentially wild type, then there is no effective restriction on reproduction. Other individuals, such as are described below, may carry traits that affect future generations in some way, preferably rendering them unfit.

As used herein, "unfit" and associated terms can refer to simple lethality, or can otherwise refer to characteristics which, while not killing the insect, render it substantially unfit for reproduction, such as blindness, flightlessness, and sterility, including sexual transformation, or any other property or characteristic which renders them uncompetitive or less than fully vigorous under at least some circumstances.

The term "fitness", as used herein, generally relates to the numbers of viable progeny able to be produced in the next generation compared to the wild type, which is

deemed to have a relative fitness of 1, in the absence of any selective agent. Considerations of fitness can be complicated, for example, where a dominant lethal gene is inherited, killing some of a generation and not others. Those that live may be 100% fit, but overall, or average, fitness may be considered as being 50% if half of the next generation is killed as a result of inheriting the lethal gene. Some heterozygotes, for example, may have fitness levels as high as 0.99 and occasionally as high as 1, whereas the homozygote resistant types may only have a fitness level of 0.5, or lower, depending on the nature of the resistance gene. Traits having fitness levels of less than 1 are particularly preferred targets for the present invention.

The wild type counterpart will generally be the, or a, gene found in the wild which has no, or not as great, a fitness penalty in the absence of the toxin against which it is desired to reduce, eliminate or prevent the spread of resistance. In an alternative aspect, the present invention provides a method for the inhibition or reversal of the spread of a genetic trait in a non human population capable of sexual reproduction, the method comprising introducing sexually compatible individuals substantially homozygous for a counterpart of the trait into the target population, the trait conferring a reduced level of fitness on individuals carrying that trait in either the heterozygous or homozygous form compared to individuals homozygous for the counterpart. The heterozygotes may suffer no relative fitness penalty to the wild type, or individual homozygous for allele with a higher fitness, but will generally have reduced fitness compared with individuals homozygous for resistance, when in the presence of toxin. This reduced fitness in the presence of toxin will reduce the gene pool for resistance, which is topped up by the wild type, or sensitive, genes in the released individuals, thereby reducing or preventing the spread of resistance. Resistance can even be eliminated by this method.

The counterpart may be a homologue or homologues of the gene or genes for the trait, or may be a replacement therefor, including an inactive gene or deletion, where this confers a higher fitness and eliminates the trait when the individual is homozygous therefor. The counterpart should preferably be located at the same place on the chromosome as that of the gene for the undesirable trait, although this is not essential.

It will be appreciated that the target population may be vegetable, or non human animal and, that while the target population referred to herein will generally be referred to as "insects", it will be understood that the use of this and related terms equally applies to other types of population suitable for targeting by the method of the present invention. Any suitable traits may be targeted by the method of the present invention and, while pesticide resistance is a significant target, and one that is particularly preferred, tolerance or susceptibility to environmental conditions such as chemicals, temperature etc, or to biological control agents such as predators, viruses, other parasites and parasitoids, and other traits, such as abnormalities in growth, breeding or appearance, for example, may also be targeted.

A trait which confers no selective disadvantage cannot actually be eliminated by the method of the present invention, although it may be diluted, but spread will always be beneficially slowed relative to existing strategies. The method may also be slow to yield results in populations which have significant time delays between generations. It is preferred, therefore, for populations targeted by the method of the present invention to have at least an annual breeding cycle, and preferably to have two or more breeding cycles in a year.

It is a particular advantage of the present invention that any form of resistance can be mitigated, including resistance to the method of the present invention. The effect is particularly notable when the homozygote and, especially the heterozygote, suffers a fitness penalty, in the absence of the compound to which resistance is conferred, compared to the wild type.

The methods of the invention have the particular advantage that multiple resistances can be overcome at once, even where any particular resistance is unknown or not recognised, as, provided the wild type gene is present, then it will dilute any resistance gene present. Any resistance may be targeted, even resistance to the methods of the invention.

Likewise, use of the methods of the invention will generally also result in the suppression of the spread of any new mutation that may occur, where such mutation has reduced fitness. Mutations without a fitness penalty will still be diluted, however.

The present invention is as applicable to complex resistance, involving two or more genes, as to simple resistance involving only one locus. It is only necessary for the individuals released into the target population to be homozygous for one wild type, or reduced fitness penalty, gene. However, it is preferred that the released individuals are homozygous for the wild type, or reduced fitness penalty, counterparts of 2, 3, or more, and preferably all, of any complex resistance, or trait, that may be observed.

It will be appreciated that the terms "resistance" and "trait" are used interchangeably herein, and that any reference to the term "resistance", or equivalent language, incorporates reference to any suitable trait targetable by the present invention, unless otherwise apparent.

Suitable toxins against which resistance might be raised include both naturally occurring and manufactured pesticides and control agents, such as growth control or suppressing agents, and include naturally expressed toxins, such as Bt toxin, whether the toxin is heterologous or homologous to the crop. Any form of genetically encoded resistance is targetable by the present invention.

The method of the present invention may be used in combination with any other method used for controlling or affecting a population. For example, it is advantageous to employ refugia with the methods of the invention, in order to allow wild type, or "sensitive", genes to increase in frequency. The use of refugia is particularly preferred where the substance for which resistance is sought to be reduced, prevented or eliminated is present in high doses and there is no other natural food source for the pest and/or in circumstances where the resistance allele is at a significant fitness disadvantage relative to wild type in such a refuge.

In general, only small refugia will be necessary for optimal conditions, and it will frequently be sufficient to use no deliberately planted or provided refugia at all,

GBP87955

such as where alternative food sources are available, such as domestic plantings, or where there is a significant influx of non-resistant individuals.

Refugia, where used, may be of any appropriate size, as determined by those skilled in the art. As a guide, suitable sizes, expressed as a percentage of total planting area, are between 0.1% and 50%, more preferably between 1 and 20%, with a size of about 5% being a preferred size.

The size of any refugia will also be dependent on location and cluster size. For example, it may be sufficient simply to mix 5% non-Bt seeds with a supply of Bt seeds, where the method is to combat the spread of Bt resistance. Where a refuge is distinct from the main planting, then a size of 10%, or greater, may be desirable.

Individuals for release into target populations are preferably entirely homozygous for the wild type counterpart of the resistance gene, but it will be appreciated that there is some possibility that a low level of the resistance gene may be present in the cultivated individuals. This is generally undesirable, and can be substantially completely eliminated by the appropriate choice of breeding stock and breeding conditions which should, preferably, entail the complete absence of the toxin appropriate to the resistance gene.

Methods for monitoring the cultivated stocks may also be used to ensure that the resistance gene is kept at minimal, or preferably zero levels.

In general, it is undesirable to release large quantities of wild type pests, even if the end result is to eliminate resistance to a particular pesticide. Large quantities of mosquitoes, for example, or crop devouring pests will often not be seen as being advantageous.

Surprisingly, it has been established that release of individuals, or insects, with dominant lethal genes, but which carry the wild type counterpart of the present invention for the target trait, not only controls, or tends to control, the population, but prevents, reduces or reverses the incidence of resistance in the population.

Thus, in an alternative aspect, there is provided a method as described above, wherein the individuals released into the target population carry a dominant lethal trait, or trait which results in reduced fitness in at least some individuals in at least one subsequent generation. 100% lethality in the F_1 generation is not preferred.

It is particularly preferred that the sensitive, or wild type, gene not be appreciably linked to the dominant lethal gene. Thus, as the gene is carried through the generations, the resistance trait will become associated with the dominant lethal in up to half of the individuals carrying the dominant lethal, so that copies of the gene will be eliminated in subsequent generations where the lethal kills the progeny, such as all of the female progeny in the case of a 100% effective female specific dominant lethal.

In this aspect of the invention, it has been found that the effective control is increased, so that release of individuals carrying a dominant lethal gene and carrying a suitable wild type gene provides a synergistic method for controlling a pest population.

It is preferred that the lethal trait not confer 100% death rate on the immediate succeeding generation prior to any opportunity that that generation may have to sexually reproduce. It is acceptable for mortality rates to be high, possibly as much as 99%, for example, but if the wild type counterpart gene is not passed on to succeeding generations, then there will be no impact on the spread of resistance, other than through reduction in the population.

It will be appreciated, therefore, that SIT is not generally preferred as a vehicle for the wild type counterpart genes, as SIT generally results in no introgression of any genotype. However, an SIT technique wherein the individuals merely have a compromised, rather than totally eliminated, ability to reproduce may be employed.

Another suitable method to combine with the method of the present invention is that of F_1 sterility, which is of particular use with the Lepidopterans, wherein a low dose of radiation is sufficient to sterilise females but not males. However, with the correct

selection of radiation dose, the female offspring of the irradiated males will also be sterile.

In another technique, cultivated individuals raised under control conditions may be homozygous for a lethal gene and an appropriate suppressor therefor. It is generally preferred that these not be linked so that, whilst the F_1 generation will all survive, the F_2 generation will suffer 25% mortality, assuming partnering with only wild types.

More preferably, the present invention envisages the use of suppressible, or conditional, dominant lethal genes in combination with the methods of the invention.

Suitable dominant lethals are generally controlled by environmental conditions, such as temperature, diurnal rhythm or dietary components, such as tetracycline. Suitable systems which may be adapted for use in the present invention include those described by Heinrich *et al.* (PNAS (2000), *97*, vol. 15, 8229-8232) and Thomas *et al.* (Science (2000), *287*, 2474 – 2476). Pre-release construction or activation of a lethal, e.g. a female-specific lethal, by use of site-specific recombinases, such as Flp/FRT or cre/lox, is also envisaged. Thus, *in vivo* construction or activation of a lethal by use of site-specific recombinases or other DNA-modifying agents is also envisaged.

In general, it is preferred that the dominant lethal gene be selective, and it is generally preferred that the selectivity be for the female, in order to reduce the number of progeny. However, the present invention also envisages male specific dominant lethals.

In certain circumstances, it will be appreciated that it may be desirable to label either the wild type or the dominant lethal gene, in order to follow progress through the target population. Suitable labels are well known, such as dyes or phenotypic markers. It will be appreciated that such markers should be as closely linked as possible to the gene, in order to ensure that they are, indeed, marking the passage of the gene. As such, it will be appreciated that the marker should preferably be immediately adjacent the selected gene.

Individuals suitable for release into target populations may be bred by any recognised means. Where the individuals carry a dominant lethal, then this should generally be suppressed, as far as possible, during preparation of a release population, in order to maximise yield. In some circumstances, it may be appropriate to remove any suppression before release to allow the final generation to be affected, such as in the case of female specific lethals, so that only male "carriers" are released into the target population. This is particularly appropriate where the female is a pest in itself, such as with mosquitoes and medfly, for example.

Suitable populations that may be targeted include those of the following species:

Australian sheep blowfly (*Lucilia cuprina*)

New world screwworm *(Cochliomyia hominivorax)*

Old World Screwworm *(Chrysomya bezziana)*

Tsetse fly *(Glossina spp)*

Stable Fly (*Stomoxys calcitrans*)

Face Fly (*Musca autumnalis*)

Horn Fly (*Haematoba irritans*)

Asian tiger mosquito (*Aedes albopictus*)

yellow fever mosquito *(Aedes aegypti)*

malaria mosquitoes, e.g. *(Anopheles gambiae, Anopheles Stephens, Anopheles funestus, Anopheles arabiensis, Anopheles dirus)*

Other mosquito vectors of disease, e.g. (*Culex pipiens, Culex quinquefasciatus*)

Japanese beetle *(Popilla japonica)*

White-fringed beetle *(Graphognatus spp.)*

Boll weevil *(Anthonomous grandis)*

Corn Rootworms: Western (*Diabrotica virgifera virgifera*), Northern (*Diabrotica barberi*), Southern (*Diabrotica undecimpunctata howardi*) and Mexican (*D. virgifera zeae*)

Red Palm Weevil (*Rhynchophorus ferrugineus*)

Sweet potato Weevils (*Cylas formicarius, eucepes postfasciatus*)

Colorado beetle (*Leptinotarsa decemlineata*)

Pine Shoot Beetle *(Tomicus piniperda)*

Mahogany Shoot Borer (*Hypsipyla robusta*)

Flour Beetle (*Tribolium confusum*)

Pea Weevil (*Bruchus pisorum*)

Grian borers (*Prostefanus truncatus*, *Rhyzopertha dominica*)

Flat grain beetle *(Cryptolestes ferrugineus)*

Granary & Rice Weevils (*Cytophilus spp.*)

Citrus blackfly *(Aleurocanthus woglumi)*

Oriental fruit fly *(Dacus dorsalis)*

Olive fruit fly *(Dacus oleae)*

tropical fruit fly *(Dacus cucurbitae, Dacus zonatus)*

Mediterranean fruit fly *(Ceratitis capitata)*

Natal fruit fly *(Ceratitis rosa)*

Cherry fruit fly *(Rhagoletis cerasi)*

Queensland fruit fly *(Bactrocera tryoni)*

Caribbean fruit fly *(Anastrepha suspensa)*

Carambola Fruit Fly (*Bactrocera carambolae*)

Mexican Fruit Fly (*Anastrepha ludens*)

Onion Fly *(Delia antiqua)*

Mushroom flies (*Lycoriella mali, Lycoriella auripila & Megaselia spp.*)

Other fruit flies (Tephritidae)

Gypsy moth *(Lymantria dispar)*

Codling moth *(Cydia pomonella)*

Brown tail moth *(Euproctis chrysorrhoea)*

rice stem borer *(Tryporyza incertulas)*

Pink Bollworm (*Pectinophora gossypiella*)

Navel Orangeworm (*Amyelois transitella*)

Peach twig worm (*Anarsia lineatella*)

Painted Apple Moth (*Teia anartoides*)

Corn Earworm (*Helicoverpa armigera, Helicoverpa zea*)

Tobacco Budworm (*Heliothis virescens* - and other Heliothines)

Tobacco Hornworm (*Maduca sexta*)

Potato Tuber Moth (*Phthorimaea operclella*)

Date Moth (*Ectomyelois ceratoniae*)

Oriental Fruit Moth (*Grapholita molesta*)

Diamondback moth (*Plutella xylostella*)

Indian Meal Moth (*Plodia interpunctella*)

Greenhouse Whiteflies (e.g. *Bemisia tabaci, Trialeurodes vaporarium*)

Cattle Fever tick (*Boophilus microplus*) and other ticks of veterinary importance

Psocids (*Liposcelis* spp.).

Suitable quantities for release are generally calculated in accordance with the expected size of population into which they are to be released. With methods such as SIT, these sizes are typically between 5 and >100 times the size of the target population. Using the method of the present invention, with a sex specific dominant lethal and 50% refugia, it is only necessary to release an amount of individuals corresponding to 0.3, or 30%, the size of the target population to completely reverse the spread of some types of resistance. These figures depend on many factors, including relative fitnesses and refugia sizes, so that release sizes will necessarily vary, and have differing efficacies, according to such parameters. Releases of 10% and lower also have a significant effect on the spread of many types of resistance.

Size estimations will often be approximate, as population densities may be only approximately known, and it may not be apparent how far the released individuals may penetrate into the target population.

Accordingly, where there is no imminent threat of resistance developing, for example, releases of as low as 0.1%, for example, may be sufficient to prevent resistance developing, although it is generally preferred to release between 1 and 10%

for control purposes. Where there is a threat, or where the release is coupled with other control means, such as RIDL, then releases of between 10 and 10,000% are preferred, with 30 to 5,000% being more preferred, especially 50 to 1000%. Such releases can be repeated as often as necessary, typically once per generation, although other regimens will be apparent to those skilled in the art.

The values used to calculate release numbers depend on various parameters, especially the relative competitiveness (C) of the released individuals and the reproductive rate of the wild population (R_0). Neither is easy to measure, but estimates can be obtained, particularly as the program progresses. In general, it is preferred that the release rate is more, and preferably substantially more, than $(R_0-1)/C$. Relative competitiveness might typically be 0.1-0.5, while R_0 varies by species, but will generally be in the range 1.5-10. Appropriate minimum release rates may then be from 1 – 90 (100% to 9000%) for population control, but resistance management will generally be effective considerably lower rates. Population control will also be effective at lower rates where RIDL is combined with another control method, such as the use of Bt, or other toxin, which will often be the case when using the methods of the invention.

As used herein, R_0 is the maximum average reproductive rate under normal conditions, i.e. ignoring density-dependent effects, and will be reduced in the presence of a toxin, for example. If R_0 were not greater than 1 under normal conditions, the population would not expand and would be unlikely to be considered a pest.

Where reducing or eliminating resistance is referred to herein, it will be understood that this includes use of the methods of the present invention in circumstances where significant resistance already exists. For example, the frequency of a resistance allele in a population where it has already attained an economically significant level, e.g. 20%-99%, may be reduced by the release of, preferably very large, amounts of sensitive individuals. This may advantageously be in conjunction with reducing, e.g. to zero, the use of the treatment against which the resistance has developed. Use of the invention, especially where the released individuals carry a dominant lethal gene, will always tend to increase the rate at which the resistance allele

is eliminated or substantially eliminated, or reduced below an economic threshold, from a population, and can be used to reduce the frequency of the resistance allele under circumstances where, without such releases, it would instead increase in frequency.

The methods of the invention are also suitable to ensure grower compliance, as it is not necessary for the grower to be responsible for the treatment, and those skilled in the art may determine the best policy for protection against resistance developing in any given geographical locale.

It will be appreciated that the present invention extends to methods for the preparation of individuals for release, as well as to the individuals themselves, where novel.

The invention further provides a method for calculating the necessary amount of individuals required for release in order to inhibit, prevent or reverse the spread of a genetic trait, comprising the use of any one or more of the techniques and/or formulae described herein.

The invention will now be further illustrated with respect to the accompanying, non-limiting Examples, which are for purposes of illustration only.

EXAMPLE 1

A population genetics framework to examine the effects of refugia and RIDL release on the management of *Bt* resistance in a pest population

Model structure and assumptions
We assume that the pest insect population is distributed at random across a fixed crop population, a proportion Φ of which expresses *Bt* toxins. The conventional (non-*Bt*) crop refuge size is therefore 1-Φ. Within the insect population, susceptibility to the *Bt* toxins in the larval stage is assumed to be controlled by a single autosomal locus

existing as two alleles; sensitive, S, and resistant, r. Therefore, there are three genotypes in the wild population, SS, Sr and rr.

We further assume that all fitness costs associated with the S and r alleles, in Bt and non-Bt crops, operate prior to adulthood, e.g. during the larval stage. Under the assumption of a random distribution of larvae across Bt and non-Bt crops, the average relative fitness, Ω, of larvae of genotype i is

$$\Omega_i = \left[\omega_i \Phi + \upsilon_i (1 - \Phi)\right] \qquad \text{(eq. 1)}$$

where ω_i is the relative fitness of larvae in the roots of Bt crops, and υ_i is the relative fitness of larvae in non-Bt crops (which is set equal to 1 for the wild-type SS genotype).

If the frequency of the resistance r allele is p in the adult generation t, then, assuming no mutation, the change in allele frequency in generation $t+1$, Δp, is given by

$$\Delta p = \frac{p_t^2 \Omega_{rr} + p_t q_t \Omega_{Sr}}{q_t^2 \Omega_{SS} + 2 p_t q_t \Omega_{Sr} + p_t^2 \Omega_{rr}} - p_t, \qquad \text{(eq. 2)}$$

where $q_t = 1 - p_t$. A simple conceptual framework for the model is shown in Figure 1, below.

Figure 1. Simple life cycle used in the basic model of Bt selection.

One generation

Equilibrium allele frequency

Setting equation 2 equal to zero and solving which respect to p reveals there are three fixed points of resistant allele frequency, p^* at which $\Delta p=0$. These fixed points are $p^*=0$, ρ and 1, where

$$\rho = \frac{\left(\upsilon_{Sr} + \omega_{Sr}\Phi - \upsilon_{Sr}\Phi - \omega_{SS}\Phi + \Phi - 1\right)}{\left(2\upsilon_{Sr} - 2\upsilon_{Sr}\Phi + 2\omega_{Sr}\Phi + \upsilon_{rr}\Phi - \omega_{rr}\Phi - \omega_{SS}\Phi - \upsilon_{rr} + \Phi - 1\right)}. \qquad \text{(eq. 3)}$$

The stability of these equilibria is dependent on the relative fitness of the different genotypes in *Bt* (ω_i) and non-*Bt* (υ_i) crops and the proportion crops expressing *Bt* (Φ).

Stable intermediate equilibrium

Importantly, ρ only exists as a stable equilibrium lying between 0 and 1, if the proportion of *Bt* falls between a lower, Φ_1, and upper, Φ_2, critical threshold, provided $\Phi_1 < \Phi_2$.

The lower critical thresholds may be found by setting ρ equal to 0 in equation 3 and solving with respect to Φ to give

$$\Phi_1 = \frac{\left(1-\upsilon_{Sr}\right)}{\left(1-\upsilon_{Sr}+\omega_{Sr}-\omega_{SS}\right)}. \qquad \text{(eq. 4)}$$

Similarly, the upper critical thresholds may be found by setting ρ equal to 1 in equation 3 and solving with respect to Φ to give

$$\Phi_2 = \frac{\left(\upsilon_{rr}-\upsilon_{Sr}\right)}{\left(\upsilon_{rr}-\upsilon_{Sr}+\omega_{Sr}-\omega_{rr}\right)}. \qquad \text{(eq. 5)}$$

Provided $\Phi_1 < \Phi_2$, then if the proportion of *Bt* crops lies between Φ_1 and Φ_2 the frequency of the *r* allele will settle at a stable equilibrium $p^*=\rho$, which lies above zero and below 1. However, if the proportion of *Bt* crops lies below Φ_1, the frequency of the

resistant allele will decline to extinction (i.e. $p*=0$). Conversely, if the proportion of Bt crops is above Φ_2, the resistant allele will eventually go to fixation (i.e. $p*=1$).

Unstable intermediate equilibrium

The fixed point ρ is unstable, however, if $\Phi_1 \geq \Phi_2$ and so the resistant allele will eventually either go to extinction or fixation. Under the unstable conditions, the resistant allele will only spread if the frequency p is greater than ρ. We can, therefore derive a third threshold for the proportion of Bt crops, Φ_3, which determines whether the resistant allele will go to fixation or extinction, under the condition that $\Phi_1 \geq \Phi_2$. By setting $p=\rho$ in equation 3 and solving with respect to Φ we can show that

$$\Phi_3 = \frac{\left(\upsilon_{Sr} - 2p\upsilon_{Sr} + p\upsilon_{rr} + p - 1\right)}{\upsilon_{Sr} - \omega_{Sr} + \omega_{SS} + p - 2p\upsilon_{Sr} - p\omega_{rr} + p\upsilon_{rr} + 2p\omega_{Sr} - p\omega_{SS} - 1}. \qquad \text{(eq. 6)}$$

So when the equilibrium frequency ρ is unstable (i.e. when $\Phi_1 \geq \Phi_2$), the resistant allele will spread to fixation if the proportion of Bt crops is greater than Φ_3. If $\Phi<\Phi_3$, the frequency of resistant allele will decline to extinction. Finally, if $\Phi=\Phi_3$ the frequency will remain constant, although slight perturbations in Φ above or below the critical threshold Φ_3 will result in fixation or extinction, respectively, of the r allele.

Complete recessiveness

If $\omega_{Sr}=\omega_{SS}$ (or $\omega_{Sr}=\omega_{SS}=0$) and $\upsilon_{Sr}=\upsilon_{SS}=1$, then again fixation determined with respect to Φ_3. In this special case of complete recessiveness in the phenotypes expressed in both Bt and non-Bt crops, then $\Phi_2=\Phi_3$. We can show this by substituting in equation 5 to give

$$\Phi_2 = \frac{1-\upsilon_{rr}}{\left(1-\upsilon_{rr}\right)+\left(\omega_{rr} - \omega_{Sr}\right)} \qquad \text{(eq. 7)}$$

Similarly we can substitute into equation 6 to give

$$\Phi_3 = \frac{1-2p+p\upsilon_{rr}+p-1}{1-2p+p\upsilon_{rr}+p-1+2p\omega_{SS}-p\omega_{SS}-\omega_{SS}+\omega_{SS}-p\omega_{rr}} \qquad \text{(eq.8)}$$

which simplifies to

$$\Phi_3 = \frac{1 - \upsilon_{rr}}{[(1 - \upsilon_{rr}) + (\omega_{rr} - \omega_{SS})]}$$
(eq. 9)

This can be intuitively interpreted as the difference in fitness between the wild-type and homozygous recessive in non-*Bt* crops, divided by the magnitude of the sum of fitness differences in both *Bt* and non-*Bt* crops.

Complete dominance

Note, if $\omega_{Sr} = \omega_{rr}$ and $\upsilon_{Sr} = \upsilon_{SS}$ then fixation determined with respect to Φ_3. In the special case of complete dominance in the phenotypes expressed in both *Bt* and non-*Bt* crops, then $\Phi_1 = \Phi_3$. Equation 4 cannot be simplified, but as you can see equals the difference in fitness between the wild-type and the resistant genotypes in non-*Bt* crops, divided by the magnitude of the sum of fitness differences in both *Bt* and non-*Bt* crops. By substituting in equation 6 we get

$$\Phi_3 = \frac{(\upsilon_{rr} - 2p\upsilon_{rr} + p\upsilon_{rr} + p - 1)}{\upsilon_{rr} - \omega_{rr} + \omega_{SS} + p - 2p\upsilon_{rr} - p\omega_{rr} + p\upsilon_{rr} + 2p\omega_{rr} - p\omega_{SS} - 1}.$$
(eq. 10)

which simplifies to give

$$\Phi_3 = \frac{1 - \upsilon_{rr}}{[(1 - \upsilon_{rr}) + (\omega_{rr} - \omega_{SS})]}.$$
(eq. 11)

Equivalence of threshold condition

Note, under the complete extremes of recessiveness and dominance, the threshold for fixation/extinction, Φ_3, is the same. So provided $\Phi > \Phi_3$, the r allele will go to fixation regardless of whether the allele is fully recessive or fully dominant. However, the time taken to reach fixation will depend on the magnitude of Φ.

Determinants of ρ equilibrium stability

If the r allele is fully recessive with respect to the resistant phenotype, then $\omega_{Sr} = \omega_{SS}$ and so the lower threshold condition Φ_1 (equation 4) equals 1 (provided $\upsilon_{Sr} < \upsilon_{SS}$) resulting in $\Phi_1 \geq \Phi_2$ always being satisfied. Thus, recessiveness results in an unstable ρ and the resistant allele will go to fixation if the proportion Bt exceeds the critical threshold Φ_3.

If the r allele is dominant with respect to the resistant phenotype, then $\omega_{Sr} = \omega_{rr}$ and so the upper threshold Φ_2 (equation 5) equals 1 (provided $\upsilon_{Sr} > \upsilon_{rr}$) and the lower limit Φ_1 is less that 1 (as $\omega_{Sr} > \omega_{SS}$ by the definition of resistance) resulting in $\Phi_1 < \Phi_2$ always being satisfied. Thus, dominance always results in a stable ρ and the resistant allele will only to fixation if the proportion Bt exceeds the critical threshold Φ_2.

Finally, if the r allele is partially dominant with respect to the resistant phenotype, then $\omega_{SS} < \omega_{Sr} < \omega_{rr}$ and either $\Phi_1 < \Phi_2$ or $\Phi_1 \geq \Phi_2$ may be satisfied, and so an intermediate stable equilibrium may, or may not, arise.

Note, high-dose refugia strategy, equates to recessive model in terms of resistant phenotype of heterozygote, i.e. $\omega_{Sr} = \omega_{SS}$. So ρ is unstable and resistance will go to fixation if $\Phi > \Phi_3$. However, a low-dose strategy, equates to partial dominance model, i.e. $\omega_{SS} < \omega_{Sr} < \omega_{rr}$. And so depending on the details can get $0 < \rho^* < 1$.

EXAMPLE 2

RIDL release

We assume that mass-reared males are released at a fixed ratio to the wild type population each generation (such that a proportion, α, of the total population each generation is made up of released males). All male, engineered released males are assumed to be homozygous sensitive to *Bt* toxins, and also homozygous at one autosome for the RIDL construct. The transformed autosome containing RIDL is termed L, equivalent to untransformed autosome w in the wild population (see Figure 2), which we assume is different to the autosome carrying the toxin sensitive locus (i.e. RIDL and *Bt* resistant loci are unlinked).

We assume the RIDL construct also imposes a sex-specific fitness cost during the larval stage inside the roots. Under the assumption of a random distribution of larvae across *Bt* and non-*Bt* crops, the average relative fitness, Ω, of larvae of genotype *i* is

$$\Omega_i = (1 - \varepsilon_i)[\omega_i \Phi + \upsilon_i (1 - \Phi)]$$

where ε_i is the sex-specific, relative fitness cost imposed by RIDL and ω_i and υ_i are the relative fitness of larvae in *Bt* and non-*Bt* crops, respectively

If RIDL were fully dominant lethal, then for all female larvae containing RIDL $\varepsilon_i=1$ and so average fitness $\Omega_i=0$. Under these conditions, as shown in Figure 2 and Figure 3, there are a total of seven male and three female genotypes (defined at the w/L autosome and *S/r* locus). Nine (six male and three female) genotypes resulting from matings in the field, while the tenth (LL*SS*) is the engineered RIDL release male. (Note, we can relax the $\varepsilon=1$ assumption at the laval stage for females to allow some viable RIDL females to emerge; this obviously increases the number of possible genotypes up to 18 - ww*SS*, ww*Sr*, ww*rr*, Lw*SS*, Lw*Sr*, Lw*rr*, LL*SS*, LL*Sr*, LL*rr* both female and male).

The structure of the RIDL release model is essentially the same as that shown in Figure 1 but adapted to include the different viable genotypes as shown in Figure 3.

For any given set of assumptions about the relative fitness of the different genotypes on Bt-crops (ω_i) and non-Bt crops (υ_i), the fitness costs associated with RIDL in the larval stage (ε_i) and the starting frequency of the resistant allele (p_0) we can compare the dynamics of the r allele frequency with and without RIDL release, for any combination of the proportion of crops expressing Bt (Φ) and the release RIDL frequency (α).

Model outputs

Releasing RIDL males always reduces the rate of spread of the r allele, even at very low release ratios (e.g. 1 RIDL release for every 10 wild types). We can formally quantify the effects of RIDL release strategies relative to a refuge only approach, by using the parameters defined by Carrière & Tabashnik (Carrière, Y & Tabashnik, BE. 2001. Reversing insect adaptation to transgenic insecticidal plants. Proc. Roy. Soc. (Lond) B. 1475-80) in their examination of the effect of refugia on the management of resistance in agricultural pest populations. The results are shown in Figures 4 and 5 and Tables 2, 3 and 4.

Table 1. Parameter values used in quantifying the model outputs that allow comparison with the findings of Carrière & Tabashnik (2001).

Variable	Value
Starting r allele frequencies in generation 0	
p_0	0.1
Relative fitness on non-Bt crops (υ)	
υ_{SS}	1
υ_{Sr}	1
υ_{rr}	0.7 or 0.4
Relative fitness on Bt crops (ω)	
ω_{SS}	0 or 0.01
ω_{Sr}	0 or 0.01
ω_{rr}	0.1, 0.2 or 0.4
Proportion Bt crops	
Φ	0.5, 0.7, 0.9 or 0.99
Sex-specific RIDL fitness cost in larvae (ε)	
ε_{male}	0.1
ε_{female}	1

25

Figure 2. Genotypes in the RIDL-release model.

Total of 7 male (1 engineered release type, 3 wild-types and 3 F2 types) and 3 female (all wild-types) genotypes

Male (1) engineered genotype for release

Male (3) and Female (3) "wild-types"

Male (3) F2s

GBP87955

26

Figure 3. RIDL-release refugia model.

Generation	Male		Female	

F0 adults   wwSS wwSr wwrr LLSS (mass release)   wwSS wwSr wwrr

F0 gametes   wS   wr   LS   wS   wr

Random mating

F1 zygotes/larvae   wwSS wwSr wwrr LwSS LwSr   wwSS wwSr wwrr LwSS LwSr

Fitness costs imposed by resistance alleles, Bt toxin and RIDL construct.
Average relative fitness, Ω, of genotype i: $\Omega_i = (1-\varepsilon_i)\,[\omega_i\,\Phi + \upsilon_i(1-\Phi)]$

F1 adults   wwSS wwSr wwrr LwSS LwSr LLSS   wwSS wwSr wwrr

F1 gametes   wS   wr   LS   Lr   Random mating   wS   wr

F2 zygotes/larvae   wwSS wwSr wwrr LwSS LwSr Lwrr   wwSS wwSr wwrr LwSS LwSr Lwrr

Fitness costs imposed by resistance alleles, Bt toxin and RIDL construct.
Average relative fitness, Ω, of genotype i: $\Omega_i = (1-\varepsilon_i)\,[\omega_i\,\Phi + \upsilon_i(1-\Phi)]$

F2 adults   wwSS wwSr wwrr LwSS LwSr Lwrr LLSS   wwSS wwSr wwrr

F2 gametes   wS   wr   LS   Lr   Random mating   wS   wr
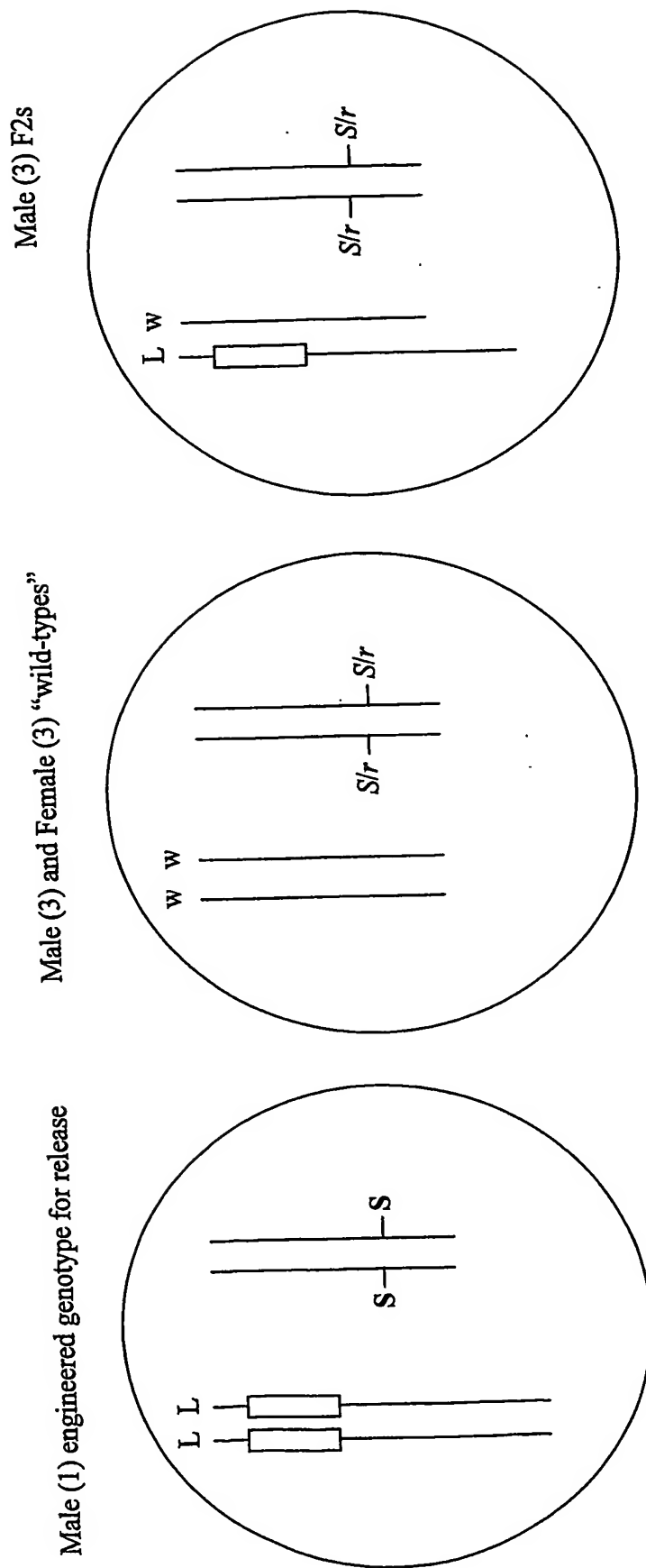
Figure 4. Example of the effects of a 1:10 RIDL release ratio (i.e. 1 RIDL to 10 wild-types) and on the evolution of resistance. The parameter values are shown in Table 1, with $\upsilon_{Sr} = 1$, $\upsilon_{rr} = 0.4$, $\omega_{rr} = 0.1$, and a 10% refuge (i.e. $\Phi = 0.9$). It can be seen that the frequency of the r allele reaches 0.5 in 36 generations in the refugia only scenario (as also predicted Carrière & Tabashnik, 2001). However, under the RIDL release scenario, even at a release ratio of 1:10, the spread of resistance is reversed.

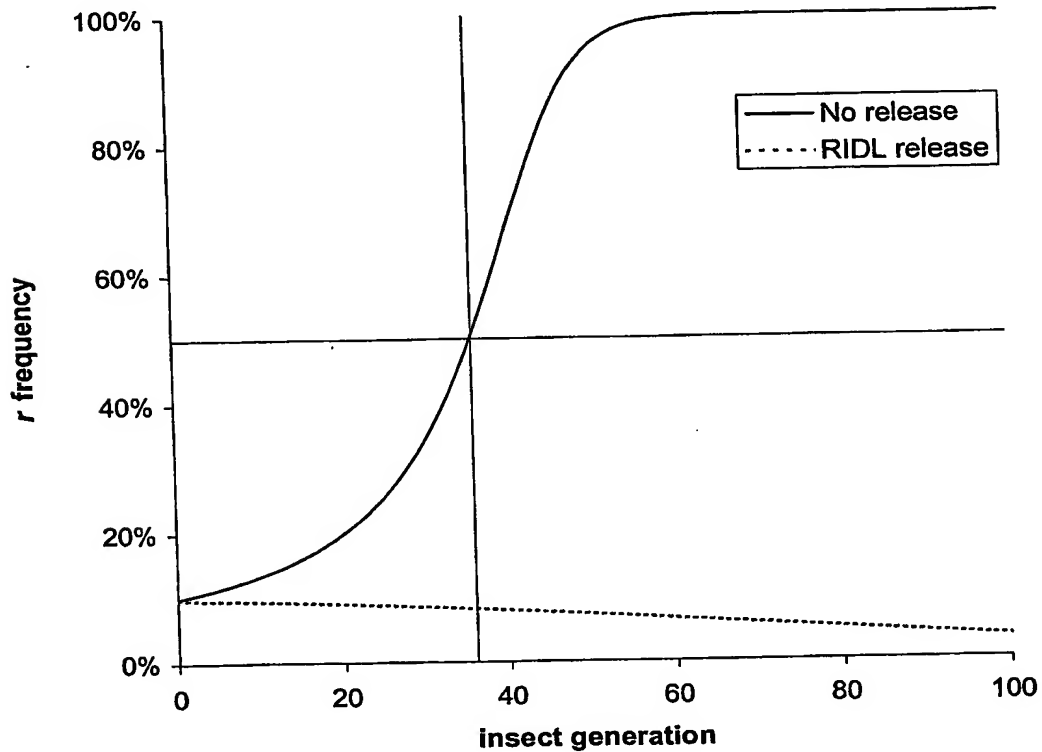**Table 2.** Effect of a 1:10 release ratio per generation on the number of generations required for the r allele frequency to reach 0.5. Note, ∞ represents a decrease in the frequency of the r allele, i.e. a reversal of resistance. As with Carrière & Tabashnik, we assume that resistance was recessive (i.e. fitness of $\upsilon_{SS}=\upsilon_{Sr}=1$ and $\omega_{SS}=\omega_{Sr}=0$). Other values, unless indicated below, are as in Table 1.

υ_{rr}	ω_{rr}	Refuge, (1-Φ)					
		0.1		0.3		0.5	
		Current	RIDL	Current	RIDL	Current	RIDL
0.7	0.1	19	31[*]	∞	∞	∞	∞
0.7	0.2	9	11[*]	64	∞	∞	∞
0.7	0.4	5	6[*]	18	28[*]	104	∞
0.4	0.1	36	∞	∞	∞	∞	∞
0.4	0.2	11	13[*]	∞	∞	∞	∞
0.4	0.4	5	6[*]	33	∞	∞	∞

[*]Note, r allele increases to a non-zero stable equilibrium level less than 1.

Table 3a and b. Effect of RIDL release at different release ratios on the number of generations required for the r allele frequency to reach 0.5 with (a) 10%, i.e. $\Phi=0.9$ and (b) 5%, i.e. $\Phi=0.95$ refuge. Other parameter assumptions are as in Tables 2.

(a)

v_{rr}	ω_{rr}	RIDL release ratio (RIDL males : wild-types)					
		0	1:10	1:5	1:1	10:1	50:1
0.7	0.1	19	31*	80*	∞	∞	∞
0.7	0.2	9	11*	12*	∞	∞	∞
0.7	0.4	5	6*	6*	9*	∞	∞
0.4	0.1	36	∞	∞	∞	∞	∞
0.4	0.2	11	13*	16*	∞	∞	∞
0.4	0.4	5	6*	7*	10*	∞	∞

*Note, r allele increases to a non-zero stable equilibrium level less than 1.

(b)

v_{rr}	ω_{rr}	RIDL release ratio (RIDL males : wild-types)					
		0	1:10	1:5	1:1	10:1	50:1
0.7	0.1	9	10*	12*	∞	∞	∞
0.7	0.2	5	5*	6*	9*	∞	∞
0.7	0.4	3	3*	4*	5*	9*	13*
0.4	0.1	10	12*	15*	∞	∞	∞
0.4	0.2	5	6*	6*	9*	∞	∞
0.4	0.4	3	4*	4*	5*	9*	13*

*Note, r allele increases to a non-zero stable equilibrium level less than 1.

Table 4. Effect of RIDL release at different on the number of generations required for the *r* allele frequency to reach 0.5 when the refuge size is only 1% (or Φ=0.99), which may arise when all agricultural production in an area is with *Bt* crops but there is migration of pest of from outside the area, or some use of alternate host plants. We also assume that there is some small probability of survival of wild-type and heterozygous pest on transgenic crops (i.e. ω_{SS}=ω_{Sr}=0.01). Other parameter assumptions are as in Tables 2. The results show the dilution effects of the RIDL release in the absence of a significant refuge. Note, significant effects on the growth of resistance are only seen at high release ratios at which point the RIDL-release strategy would be expected to have a major suppressive effect on the pest population. Also note that we are here analysing allele frequency; a highly effective *Bt* crop, especially when combined with RIDL releases, will also have a very strong effect on pest population size. This strong local suppression of the wild population is unlikely to affect the immigration rate as strongly, so the effective refuge size may increase, leading to a situation closer to that illustrated in Tables 3a and 3b.

υ_{rr}	ω_{rr}	RIDL release ratio (RIDL males : wild-types)					
		0	1:10	1:5	1:1	10:1	50:1
0.7	0.1	4	5*	5*	7*	19*	>250
0.7	0.2	3	3*	3*	4*	7*	10*
0.7	0.4	2	2*	2*	3*	4*	6*
0.4	0.1	4	5*	5*	7*	20*	∞
0.4	0.2	3	3*	3*	4*	7*	10*
0.4	0.4	2	2*	2*	3*	4*	6*

*Note, *r* allele increases to a non-zero stable equilibrium level less than 1.

Releasing RIDL males always reduces the rate of spread of the r allele, even at very low release ratios. For example, given the following parameter value assumptions:

| Variable	Value
Starting r allele frequencies in generation 0	
p_0	0.05
Relative fitness of larvae feeding on non-*Bt* crops (υ)	
υSS	1
υ_{Sr}	0.99
υ_{rr}	0.7
Relative fitness of larvae feeding on *Bt* crops (ω)	
ω_{SS}	0.01
ω_{Sr}	0.1
ω_{rr}	0.5
Proportion *Bt* crops	
Φ_{Bt}	0.5
Sex-specific RIDL fitness cost in larvae (ε)	
ε_{male}	0.1
ε_{female}	1

Figure 5 shows the effects of different values of RIDL release (α). Note that, in this Figure, α is defined as the proportion of the total adult population that is made up of newly-released individuals, so:

$\alpha=0.1$ means 1 RIDL release for every 9 wild type, equivalent to a release ratio of 1:9 in the above examples;

$\alpha=0.2$ means 2 RIDL release for every 8 wild type, or 1:4 ratio;

$\alpha=0.3$ means 3 RIDL release for every 7 wild type, or 3:7 ratio.

Figure 5. The effects of different values of RIDL release (α, as defined above).
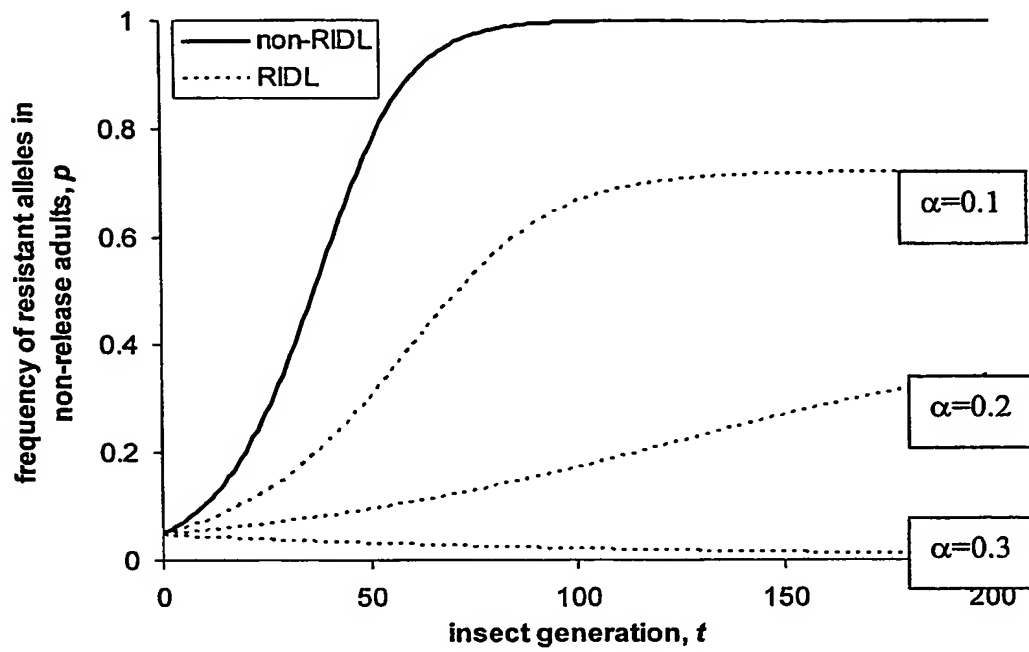
Claims:

1.   A method for the inhibition or reversal of the spread of a non wild type genetic trait in a non human population capable of sexual reproduction, the method comprising introducing sexually compatible individuals substantially homozygous for a wild type counterpart of the trait into the target population.

2.   A method according to claim 1, wherein the trait confers a reduced level of fitness on individuals carrying that trait in either the heterozygous or homozygous form, in the absence of selective conditions for the trait.

3.   A method for the inhibition or reversal of the spread of a genetic trait in a non human population capable of sexual reproduction, the method comprising introducing sexually compatible individuals substantially homozygous for a counterpart of the trait into the target population, the trait conferring a reduced level of fitness on individuals carrying that trait in either the heterozygous or homozygous form compared to individuals homozygous for the counterpart.

4.   A method for the inhibition or reversal of the spread of a genetic trait in a non human population capable of sexual reproduction, the method comprising introducing sexually compatible individuals substantially homozygous for a counterpart of the trait into the target population, wherein the individuals released into the target population carry a dominant lethal trait, or trait which results in reduced average fitness in at least one subsequent generation, said fitness preferably not being zero for the F_1 generation.

5.   A method according to claim 4, wherein the trait confers a reduced level of fitness on individuals carrying that trait in either the heterozygous or homozygous form.

6.   A method for the inhibition or reversal of the spread of a non wild type genetic trait in a non human population capable of sexual reproduction, the trait conferring a reduced level of fitness of the individuals carrying that trait in either the heterozygous or homozygous form, the method comprising introducing sexually compatible individuals substantially homozygous for the wild type counterpart of the trait into the target

population, wherein the individuals released into the target population carry a dominant lethal trait, or trait which results in reduced fitness in at least one subsequent generation.

7.   A method according to any preceding claim, wherein the heterozygote form has an associated reduced level of fitness by comparison with the homozygotes in the presence of a toxin, wherein the trait is resistance to the said toxin.

ABSTRACT

RESISTANCE DILUTION

Toxin resistance can be inhibited or reversed by introducing sexually compatible individuals substantially homozygous for the sensitive allele into the target population.

existing as two alleles; sensitive, S, and resistant, r. Therefore, there are three genotypes in the wild population, SS, Sr and rr.

We further assume that all fitness costs associated with the S and r alleles, in Bt and non-Bt crops, operate prior to adulthood, e.g. during the larval stage. Under the assumption of a random distribution of larvae across Bt and non-Bt crops, the average relative fitness, Ω, of larvae of genotype i is

$$\Omega_i = \left[\omega_i \Phi + \upsilon_i(1-\Phi)\right] \qquad \text{(eq. 1)}$$

where ω_i is the relative fitness of larvae in the roots of Bt crops, and υ_i is the relative fitness of larvae in non-Bt crops (which is set equal to 1 for the wild-type SS genotype).

If the frequency of the resistance r allele is p in the adult generation t, then, assuming no mutation, the change in allele frequency in generation t+1, Δp, is given by

$$\Delta p = \frac{p_t^2 \Omega_{rr} + p_t q_t \Omega_{Sr}}{q_t^2 \Omega_{SS} + 2 p_t q_t \Omega_{Sr} + p_t^2 \Omega_{rr}} - p_t, \qquad \text{(eq. 2)}$$

where $q_t = 1 - p_t$. A simple conceptual framework for the model is shown in Figure 1, below.

Figure 1. Simple life cycle used in the basic model of Bt selection.
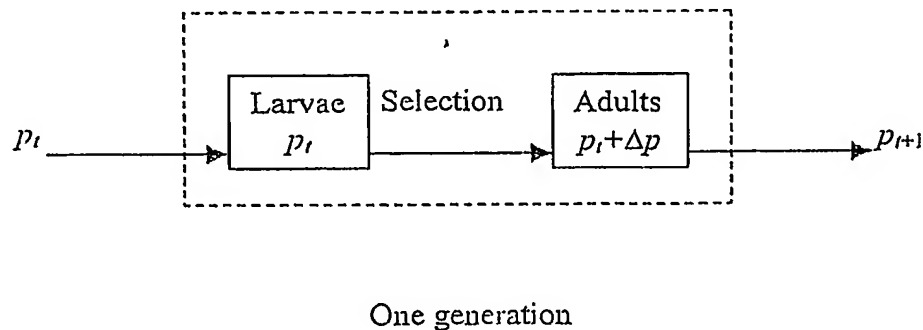

One generation

Figure 2. Genotypes in the RIDL-release model.

Total of 7 male (1 engineered release type, 3 wild-types and 3 F2 types) and 3 female (all wild-types) genotypes
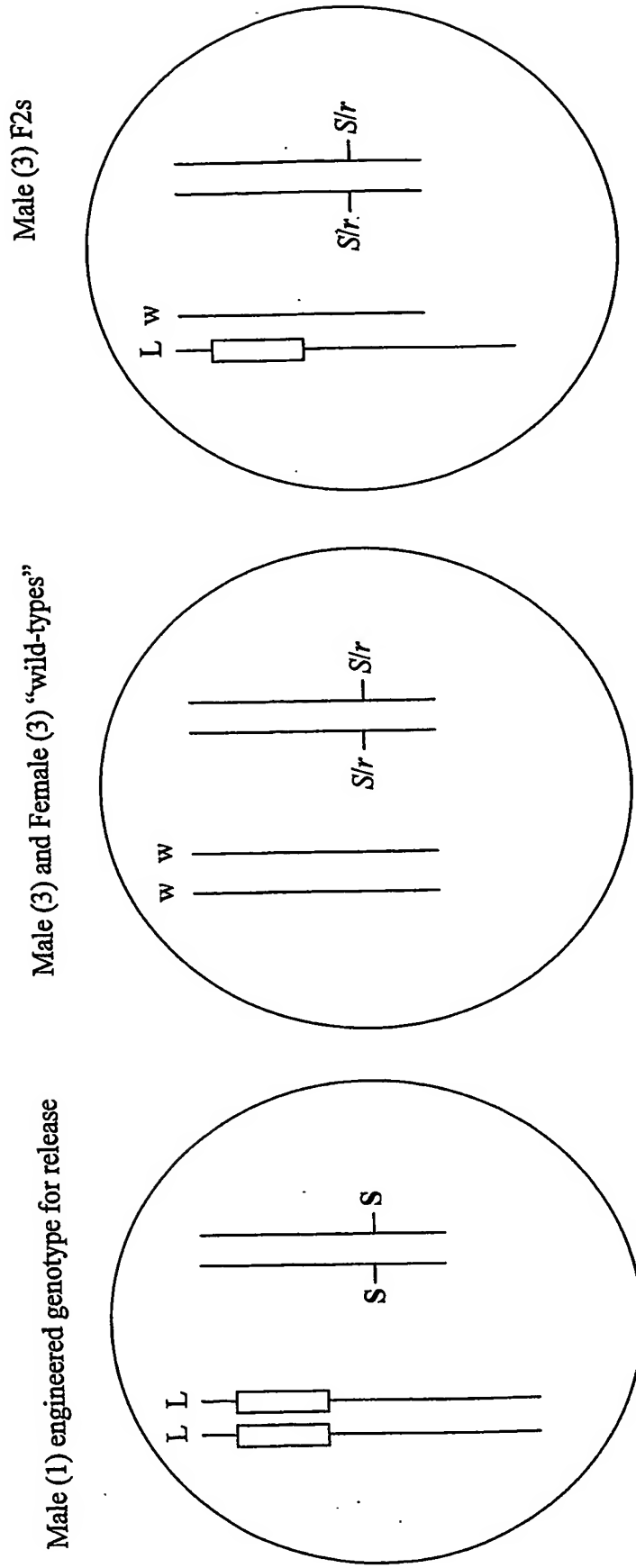
Male (1) engineered genotype for release

Male (3) and Female (3) "wild-types"

Male (3) F2s

25

GBP87955

Figure 3. RIDL-release refugia model.

26

Generation	Male	Female

F0 adults Male: *wwSS wwSr wwrr* **LLSS** (mass release)   Female: *wwSS wwSr wwrr*

F0 gametes Male: *wS   wr   LS*   Female: *wS   wr*

Random mating

F1 zygotes/larvae Male: *wwSS wwSr wwrr* LwSS LwSr   Female: *wwSS wwSr wwrr* LwSS LwSr

Fitness costs imposed by resistance alleles, *Bt* toxin and RIDL construct.
Average relative fitness, Ω, of genotype *i*: $\Omega_i = (1-\varepsilon_i) [\omega_i \Phi + \upsilon_i (1-\Phi)]$

F1 adults Male: *wwSS wwSr wwrr* LwSS LwSr **LLSS**   Female: *wwSS wwSr wwrr*

F1 gametes Male: *wS   wr   LS   Lr*   Female: *wS   wr*

Random mating

F2 zygotes/larvae Male: *wwSS wwSr wwrr* LwSS LwSr Lwrr   Female: *wwSS wwSr wwrr* LwSS LwSr Lwrr

Fitness costs imposed by resistance alleles, *Bt* toxin and RIDL construct.
Average relative fitness, Ω, of genotype *i*: $\Omega_i = (1-\varepsilon_i) [\omega_i \Phi + \upsilon_i (1-\Phi)]$

F2 adults Male: *wwSS wwSr wwrr* LwSS LwSr Lwrr **LLSS**   Female: *wwSS wwSr wwrr*

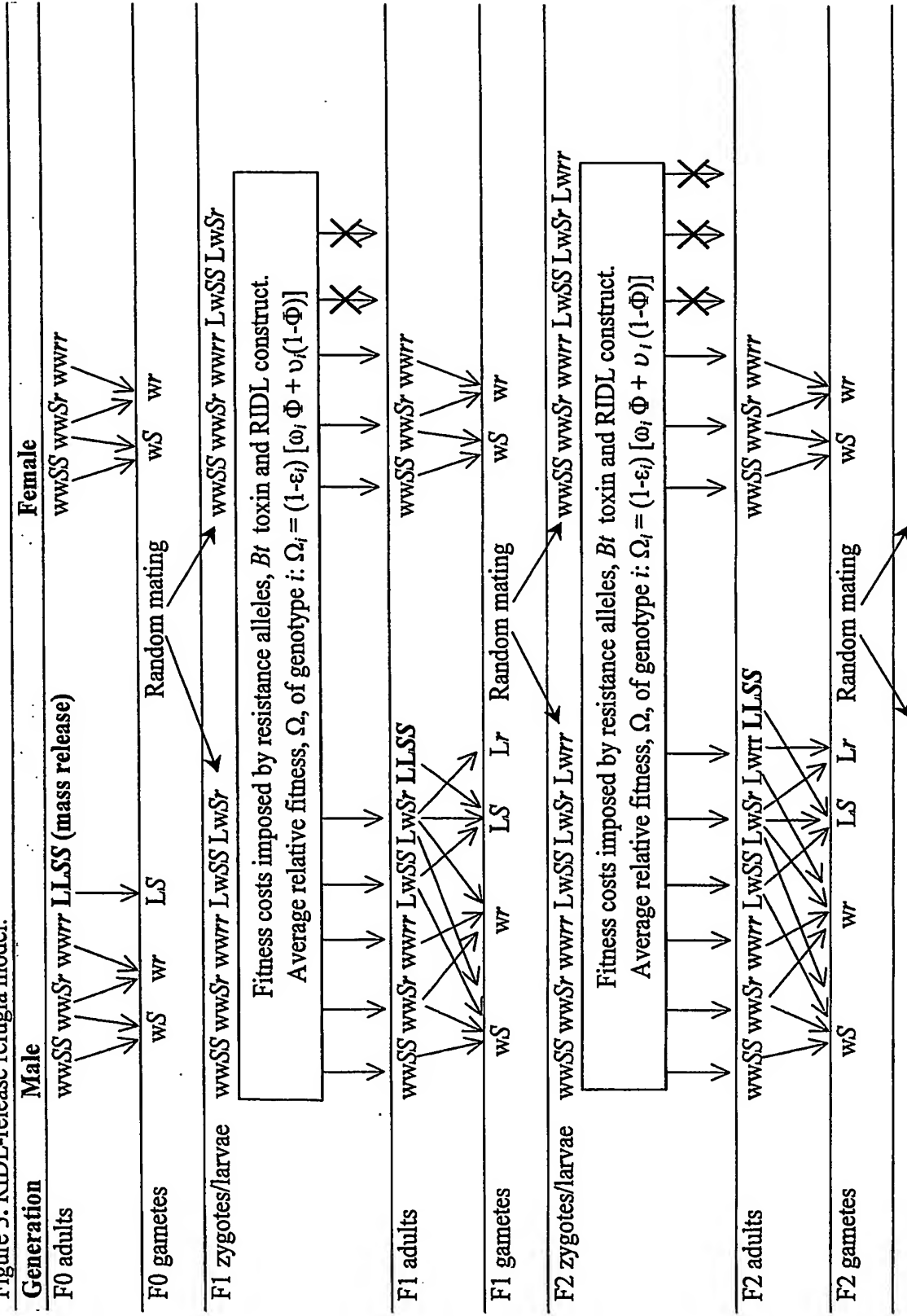**F2 gametes** Male: *wS   wr   LS   Lr*   Female: *wS   wr*

Random mating

Figure 4. Example of the effects of a 1:10 RIDL release ratio (i.e. 1 RIDL to 10 wild-types) and on the evolution of resistance. The parameter values are shown in Table 1, with υ_{Sr} =1, υ_{rr} = 0.4, ω_{rr} = 0.1, and a 10% refuge (i.e. Φ = 0.9). It can be seen that the frequency of the r allele reaches 0.5 in 36 generations in the refugia only scenario (as also predicted Carrière & Tabashnik, 2001). However, under the RIDL release scenario, even at a release ratio of 1:10, the spread of resistance is reversed.
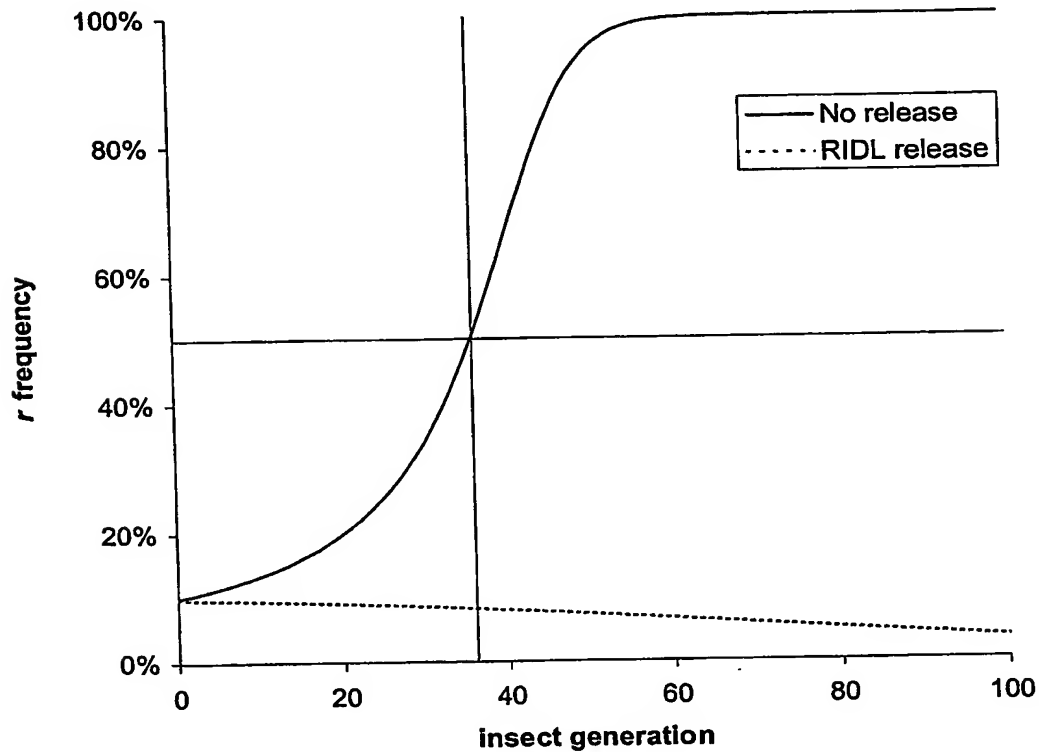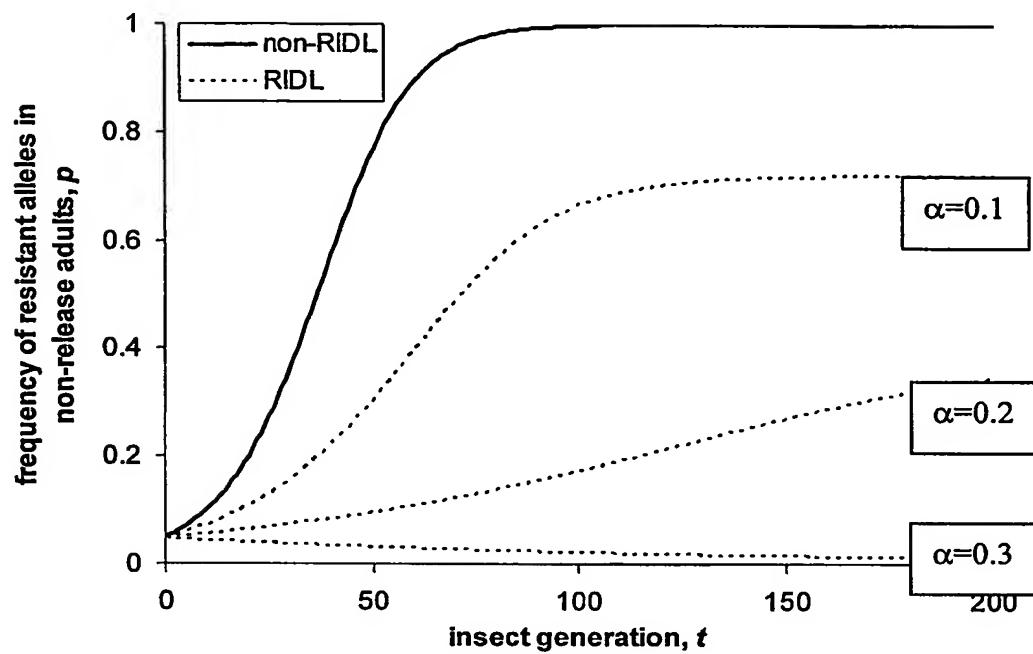
Figure 5. The effects of different values of RIDL release (α, as defined above).

PCT/GB2004/002021